WEST

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Term: L1 with hyperthermostable

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Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L4	(5756339)![pn]	2	L4
DB=USPT; PLUR=YES; OP=ADJ			
L3	5756339.pn.	1	L3
DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L2	L1 with hyperthermostable	13	L2
L1	protease	50218	L1

END OF SEARCH HISTORY

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L2: Entry 1 of 13 File: PGPB Sep 19, 2002

PGPUB-DOCUMENT-NUMBER: 20020132335
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020132335 A1

TITLE: System for expressing hyperthermostable protein

PUBLICATION-DATE: September 19, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Takakura, Hikaru	Otsu-shi		JP	
Morishita, Mio	Otsu-shi		JP	
Shimojo, Tomoko	Kyoto-shi		JP	
Asada, Kiyozo	Koka-gun		JP	
Kato, Ikunoshin	Uji-shi		JP	

US-CL-CURRENT: 435/226; 435/219, 435/252.31, 435/320.1, 435/69.1, 536/23.2

CLAIMS:

What is claimed is:

1. A gene encoding a protein consisting of an amino acid sequence in which one or more amino acid residues are deleted from the C-terminus of the amino acid sequence of SEQ ID NO: 4 and having a thermostable protease activity.

2. The protease gene according to claim 1, which encodes the amino acid sequence of SEQ ID NO:1.

3. The protease gene according to claim 2, which consists of the base sequence SEQ ID NO:2.

4. A protease gene which hybridizes with the protease gene according to claim 3 under stringent conditions and encodes a protein having a thermostable protease activity.

5. A protease gene encoding a protein consisting of an amino acid sequence in which one to several amino acid residues are deleted, substituted, inserted or added to the amino acid sequence of SEQ ID NO:1 and having a thermostable protease activity.

6. A gene encoding a n amino acid sequence represented by formula I:SIG-Ala-Gly-Gly-Asn-PRO [I]wherein SIG represents an amino acid sequence of a signal peptide derived from a subtilisin, PRO represents an amino acid sequence of a protein to be expressed.

7. The gene according to claim 6, wherein SIG is the amino acid sequence SEQ ID NO:3.

8. The gene according to claim 6, wherein PRO is an amino acid sequence of a hyperthermostable protease derived from a hyperthermophile.

9. The gene according to claim 8, wherein PRO is an amino acid sequence of a protease derived from Pyrococcus furiosus.

10. The gene according to claim 9, wherein PRO comprises the amino acid sequence of the protease consisting of an amino acid sequence in which one or more amino acid residues are deleted from the C-terminus of the amino acid sequence of SEQ ID NO:4.

11. The gene according to claim 10, which is contained in a plasmid selected from the group consisting of pSPO124 or pSPO124.DELTA.C.

12. The gene according to claim 6, wherein PRO comprises the amino acid sequence of SEQ ID NO:1.

13. A method of producing a protein, comprising: culturing a bacterium of genus Bacillus into which the gene according to claim 6 is introduced; and collecting the protein of interest from the culture.

14. The method of producing a protein according to claim 13, wherein the bacterium of genus Bacillus is Bacillus subtilis.

15. The method of producing a protein according to claim 13, wherein the gene is introduced into the bacterium of genus Bacillus by means of a plasmid vector.

16. The method of producing a protein according to claim 15, wherein a plasmid selected from the group consisting of pSPO124 or pSPO124.DELTA.C is introduced into the bacterium of genus Bacillus.

17. The method of producing a protein according to claim 15, comprising culturing Bacillus subtilis DB104/pSPO124.DELTA.C FERM P-16227, and collecting the protein of interest from the culture.

18. A plasmid vector into which the gene according to claim 6 is inserted.

19. The plasmid vector according to claim 18, selected from the group consisting of pSPO124 or pSPO124.DELTA.C.

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Search Results - Record(s) 1 through 13 of 13 returned.

☐ 1. Document ID: US 20020132335 A1

L2: Entry 1 of 13 File: PGPB Sep 19, 2002

PGPUB-DOCUMENT-NUMBER: 20020132335
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020132335 A1

TITLE: System for expressing hyperthermostable protein

PUBLICATION-DATE: September 19, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Takakura, Hikaru	Otsu-shi		JP	
Morishita, Mio	Otsu-shi		JP	
Shimojo, Tomoko	Kyoto-shi		JP	
Asada, Kiyozo	Koka-gun		JP	
Kato, Ikunoshin	Uji-shi		JP	

US-CL-CURRENT: 435/226; 435/219, 435/252.31, 435/320.1, 435/69.1, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 20020086402 A1

L2: Entry 2 of 13 File: PGPB Jul 4, 2002

PGPUB-DOCUMENT-NUMBER: 20020086402
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020086402 A1

TITLE: Hyperthermostable protease gene

PUBLICATION-DATE: July 4, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Takakura, Hikaru	Otsu-shi		JP	
Morishita, Mio	Otsu-shi		JP	
Yamamoto, Katsuhiko	Otsu-shi		JP	
Mitta, Masanori	Kyotanabe-shi		JP	
Asada, Kiyozo	Koka-gun		JP	
Tsunasawa, Susumu	Otsu-shi		JP	
Kato, Ikunoshin	Uji-shi		JP	

US-CL-CURRENT: 435/226; 435/325, 435/69.1, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 3. Document ID: US 6358726 B1

L2: Entry 3 of 13 File: USPT Mar 19, 2002

US-PAT-NO: 6358726
DOCUMENT-IDENTIFIER: US 6358726 B1

TITLE: Thermostable protease

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 4. Document ID: US 6261822 B1

L2: Entry 4 of 13 File: USPT Jul 17, 2001

US-PAT-NO: 6261822
DOCUMENT-IDENTIFIER: US 6261822 B1

TITLE: Ultrathermostable protease genes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 5. Document ID: US 6143517 A

L2: Entry 5 of 13 File: USPT Nov 7, 2000

US-PAT-NO: 6143517
DOCUMENT-IDENTIFIER: US 6143517 A

TITLE: Thermostable proteolytic enzymes and uses thereof in peptide and protein synthesis

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 6. Document ID: US 5756339 A

L2: Entry 6 of 13 File: USPT May 26, 1998

US-PAT-NO: 5756339
DOCUMENT-IDENTIFIER: US 5756339 A

TITLE: Hyperthermostable protease gene

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 7. Document ID: EP 994191 A1

L2: Entry 7 of 13 File: EPAB Apr 19, 2000

PUB-NO: EP000994191A1

DOCUMENT-IDENTIFIER: EP 994191 A1
TITLE: SYSTEM FOR EXPRESSING HYPERTHERMOSTABLE PROTEASE

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 8. Document ID: WO 9856926 A1

L2: Entry 8 of 13 File: EPAB Dec 17, 1998

PUB-NO: WO009856926A1
DOCUMENT-IDENTIFIER: WO 9856926 A1
TITLE: SYSTEM FOR EXPRESSING HYPERTHERMOSTABLE PROTEIN

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 9. Document ID: EP 870833 A1

L2: Entry 9 of 13 File: EPAB Oct 14, 1998

PUB-NO: EP000870833A1
DOCUMENT-IDENTIFIER: EP 870833 A1
TITLE: ULTRATHERMOSTABLE PROTEASE GENES

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 10. Document ID: EP 776971 A1

L2: Entry 10 of 13 File: EPAB Jun 4, 1997

PUB-NO: EP000776971A1
DOCUMENT-IDENTIFIER: EP 776971 A1
TITLE: HYPERTHERMOSTABLE PROTEASE GENE

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 11. Document ID: WO 9534645 A1

L2: Entry 11 of 13 File: EPAB Dec 21, 1995

PUB-NO: WO009534645A1
DOCUMENT-IDENTIFIER: WO 9534645 A1
TITLE: HYPERTHERMOSTABLE PROTEASE GENE

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

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☐ 12. Document ID: WO 9856926 A1 AU 9875500 A EP 994191 A1 CN 1260002 A JP 11502065 X KR 2001013540 A US 6358726 B1 US 20020132335 A1

L2: Entry 12 of 13 File: DWPI Dec 17, 1998

DERWENT-ACC-NO: 1999-080907
DERWENT-WEEK: 200271

TITLE: Recombinant hyperthermostable protease from Pyrococcus furiosus - and gene encoding it, for large scale production of the protease for industrial use.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments

KWIC | Draw Desc | Clip Img | Image

☐ 13. Document ID: WO 9534645 A1 DE 69524422 E JP 08501922 X EP 776971 A1 US 5756339 A EP 776971 A4 EP 776971 B1

L2: Entry 13 of 13 File: DWPI Dec 21, 1995

DERWENT-ACC-NO: 1996-049674
DERWENT-WEEK: 200213

TITLE: Pyrococcus furiosus hyper:thermostable protease gene - useful for recombinant prodn. of hyper:thermostable protease

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments

KWIC | Draw Desc | Image

Generate Collection | Print

Terms	Documents
L1 with hyperthermostable	13

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(FILE 'HOME' ENTERED AT 10:02:54 ON 03 JAN 2003)

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3371	FILE BIOBUSINESS
1008	FILE BIOCOMMERCE
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L5          14 DUP REM L4 (28 DUPLICATES REMOVED)
L6           1 S L5 AND (CDNA OR CLONE)
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=> d l5 ibib ab 1-14

L5 ANSWER 1 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:240396 BIOSIS
DOCUMENT NUMBER: PREV200200240396
TITLE: Thermostable **protease**.
AUTHOR(S): Takakura, Hikaru (1); Morishita, Mio; Shimojo, Tomoko; Asada, Kiyozo; Kato, Ikunoshin
CORPORATE SOURCE: (1) Otsu Japan
ASSIGNEE: Takara Shuzo Co., Ltd., Kyoto, Japan
PATENT INFORMATION: US 6358726 March 19, 2002
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Mar. 19, 2002) Vol. 1256, No. 3, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html. e-file.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB A **hyperthermostable protease** having the amino acid sequence represented by the SEQ ID NO:1 of the Sequence Listing or a sequence derived therefrom by deletion, substitution, insertion or addition of one to several amino acid residues, a gene encoding the **hyperthermostable protease**, and a process for preparing the **protease**, aiming at providing by genetic engineering techniques a hyperthermophile **protease** which is advantageous for industrial use.

L5 ANSWER 2 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:420616 BIOSIS
DOCUMENT NUMBER: PREV200100420616
TITLE: Ultrathermostable **protease** genes.
AUTHOR(S): Takakura, Hikaru (1); Morishita, Mio; Yamamoto, Katsuhiko; Mitta, Masanori; Asada, Kiyozo; Tsunasawa, Susumu; Kato, Ikunoshin
CORPORATE SOURCE: (1) Otsu Japan
ASSIGNEE: Takara Shuzo Co., Ltd., Kyoto, Japan
PATENT INFORMATION: US 6261822 July 17, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (July 17, 2001) Vol. 1248, No. 3, pp. No Pagination. e-file.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB There are provided **hyperthermostable proteases** having an amino acid sequences represented by SEQ ID Nos. 1, 3 and 5 of the Sequence Listing or functional equivalents thereof and **hyperthermostable protease** genes encoding those **hyperthermostable protease**. There is also disclosed a process for preparation of a **hyperthermostable protease** by culturing a transformant containing the gene.

L5 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:742208 CAPLUS
DOCUMENT NUMBER: 133:323312
TITLE: Protein-decomposition composition for detergents and natural rubber processing
INVENTOR(S): Takakura, Hikaru; Shimojo, Tomoko; Asada, Kiyozo; Kato, Ikunoshin
PATENT ASSIGNEE(S): Takara Shuzo Co., Ltd., Japan
SOURCE: PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000061711	A1	20001019	WO 2000-JP1996	20000330

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: JP 1999-99993 A 19990407
JP 1999-101275 A 19990408

AB The compn. is characterized by contg. a **protease** with ultrahigh heat resistance, and comprises one member selected between (1) a detergent and (2) a remover for allergenic proteins contained in a natural rubber latex. When the ingredient (1) is selected, a detergent compn. or detergent fluid is obtained which has the excellent ability to remove proteinous fouling components difficult to decomp. When the ingredient (2) is selected, a remover for allergenic proteins can be obtained with which the amt. of allergenic proteins contained in a natural rubber latex can be reduced without fail.

REFERENCE COUNT: 51 THERE ARE 51 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 4 OF 14 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 1
ACCESSION NUMBER: 1999:8129 CAPLUS
DOCUMENT NUMBER: 130:77959
TITLE: Recombinant preparation of mature form of **hyperthermostable** proteinase of Pyrococcus furiosus in Bacillus
INVENTOR(S): Takakura, Hikaru; Morishita, Mio; Shimojo, Tomoko; Asada, Kiyozo; Kato, Ikunoshin
PATENT ASSIGNEE(S): Takara Shuzo Co., Ltd., Japan
SOURCE: PCT Int. Appl., 82 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9856926	A1	19981217	WO 1998-JP2465	19980604
W: AU, CA, CN, JP, KR, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9875500	A1	19981230	AU 1998-75500	19980604
EP 994191	A1	20000419	EP 1998-923114	19980604
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
US 6358726	B1	20020319	US 1999-445472	19991208
US 2002132335	A1	20020919	US 2002-90624	20020306

PRIORITY APPLN. INFO.: JP 1997-151969 A 19970610
WO 1998-JP2465 W 19980604
US 1999-445472 A3 19991208

AB The gene encoding a **hyperthermostable protease** PFUS is isolated from Pyrococcus furiosus strain DSM3638 and used for the prodn. of 2 mature forms of **protease** by expression the gene in Bacillus. Mature forms NAPS-1 and SPO-124.DELTA.C comprised of amino

acids 133-552 and 133-544 of PFUS, resp., are prepd. by transgenic Bacillus subtilis strain DB104/pNAPS.DELTA.C and strain DB104/pSPO124.DELTA.C. Claimed are methods of recombinant prodn. of the **protease** by expression of a chimeric gene that also contains the gene encoding the signal peptide of subtilisin.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 5 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:108622 BIOSIS
DOCUMENT NUMBER: PREV200200108622
TITLE: **Hyperthermostable protease** gene.
AUTHOR(S): Mitta, M.; Yamamoto, K.; Morishita, M.; Asada, K.; Tsunasawa, S.; Kato, I.
CORPORATE SOURCE: Tsuzuki-gun Japan
ASSIGNEE: TAKARA SHUZO CO., LTD.
PATENT INFORMATION: US 5756339 May 26, 1998
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (May 26, 1998) Vol. 1210, No. 4, pp. 3553. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English

L5 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 2
ACCESSION NUMBER: 1998:699611 CAPLUS
DOCUMENT NUMBER: 130:77888
TITLE: Pyrrolidone carboxyl peptidase from the hyperthermophilic Archaeon Pyrococcus furiosus: cloning and overexpression in Escherichia coli of the gene, and its application to protein sequence analysis
AUTHOR(S): Tsunasawa, Susumu; Nakura, Satomi; Tanigawa, Tetsuo; Kato, Ikunoshin
CORPORATE SOURCE: Biotechnology Research Laboratories, Takara Shuzo Co., Ltd., Kusatsu, 525-0055, Japan
SOURCE: Journal of Biochemistry (Tokyo) (1998), 124(4), 778-783
CODEN: JOBIAO; ISSN: 0021-924X
PUBLISHER: Japanese Biochemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A gene for a pyrrolidone carboxyl peptidase (Pcp: EC 3.4.19.3, pyroglutamyl peptidase), which removes N-terminal pyroglutamyl residues from peptides and proteins, has been cloned from the hyperthermophilic Archaeon Pyrococcus furiosus using its cosmid protein library, sequenced, and expressed in Escherichia coli. The DNA sequence encodes a protein contg. 208 amino acid residues with methionine at the N-terminus. Anal. of the recombinant protein expressed in E. coli, including amino acid sequence anal. from the N-terminus by automated Edman degrdn. and ionspray mass spectrometric anal. of the peptides generated by enzymic digestions with lysyl endopeptidase and Staphylococcus aureus V8 **protease**, showed its primary structure to be completely identical with that deduced from its cDNA sequence. Comparison of the amino acid sequence of P. furiosus Pcp (P.f.Pcp) with those of bacterial Pcps revealed that a high degree of sequence identity (more than 40%) and conservation of the amino acid residues comprising the catalytic triad, Cys 142, His 166, and Glu 79. A unique short stretch sequence (positions around 175-185) that is absent in bacterial Pcps was found in P.f.Pcp. A similar stretch has also been reported recently in the amino acid sequence of Pcp from the hyperthermophilic Archaeon Thermococcus litoralis. To elucidate their contribution to the **hyperthermostability** of these enzymes, further structural studies are required.

REFERENCE COUNT: 17 THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 7 OF 14 Elsevier BIOBASE COPYRIGHT 2003 Elsevier Science B.V.
ACCESSION NUMBER: 1998265235 Elsevier BIOBASE
TITLE: Crystal structure of methionine aminopeptidase from hyperthermophile, Pyrococcus furiosus
AUTHOR: Tahirov T.H.; Oki H.; Tsukihara T.; Ogasahara K.; Yutani K.; Ogata K.; Izu Y.; Tsunasawa S.; Kato I.
CORPORATE SOURCE: T. Tsukihara, Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka 565, Japan. E-mail: tsuki@protein.osaka-u.ac.jp
SOURCE: Journal of Molecular Biology, (20 NOV 1998), 284/1 (101-124), 101 reference(s)
CODEN: JMOBAK ISSN: 0022-2836
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The structure of methionine aminopeptidase from hyperthermophile Pyrococcus furiosus (PfMAP) with an optimal growth temperature of 100.degree.C was determined by the multiple isomorphous replacement method and refined in three different crystal forms, one monoclinic and two hexagonal, at resolutions of 2.8, 2.9, and 3.5 .ANG.. The resolution of the monoclinic crystal form was extended to 1.75 .ANG. by water-mediated transformation to a low-humidity form, and the obtained diffraction data used for high-resolution structure refinement. This is the first description of a eukaryotic type methionine aminopeptidase structure. The PfMAP molecule is composed of two domains, a catalytic domain and an insertion domain, connected via two antiparallel .beta.-strands. The catalytic domain, which possesses an internal 2-fold symmetry and contains two cobalt ions in the active site, resembles the structure of a prokaryotic type MAP from Escherichia coli (EcMAP), while the structure of the insertion domain containing three helices has a novel fold and accounts for a major difference between the eukaryotic and prokaryotic types of methionine aminopeptidase. Analysis of the PfMAP structure in comparison with EcMAP and other mesophile proteins reveals several factors which may contribute to the **hyperthermostability** of PfMAP: (1) a significantly high number of hydrogen bonds and ion-pairs between side-chains of oppositely charged residues involved in the stabilization of helices; (2) an increased number of hydrogen bonds between the positively charged side-chain and neutral oxygen; (3) a larger number of buried water molecules involved in crosslinking the backbone atoms of sequentially separate segments; (4) stabilization of two antiparallel .beta.-strands connecting the two domains of the molecule by proline residues; (5) shortening of N and C-terminal tails and stabilization of the loop c.sub.3E by deletion of three residues.

L5 ANSWER 8 OF 14 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 3
ACCESSION NUMBER: 1997:779741 CAPLUS
DOCUMENT NUMBER: 128:125263
TITLE: Homology modeling of two subtilisin-like serine **proteases** from the hyperthermophilic archaea Pyrococcus furiosus and Thermococcus stetteri
AUTHOR(S): Voorhorst, Wilfried G. B.; Warner, Angela; de Vos, Willem M.; Siezen, Roland J.
CORPORATE SOURCE: Department of Microbiology, Wageningen Agricultural University, Wageningen, NL-6703 CT, Neth.
SOURCE: Protein Engineering (1997), 10(8), 905-914
CODEN: PRENE9; ISSN: 0269-2139
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The hyperthermophilic archaeon Pyrococcus furiosus produces an extracellular, glycosylated **hyperthermostable** subtilisin-like serine **protease**, termed pyrolysin (Voorhorst, W.G.B., Eggen,R.I.L., Geerling,A.C.M., Platteeuw,C., Siezen,R.J. and de Vos,W.M.

(1996) J. Biol. Chem., 271, 20426-20431). Based on the pyrolysin coding sequence, a pyrolysin-like gene fragment was cloned and characterized from the extreme thermophilic archaeon Thermococcus stetteri. Like pyrolysin, the deduced sequence of this serine **protease**, designated stetterlysin, contains a catalytic domain with high homol. with other subtilases, allowing homol. modeling starting from known crystal structures. Comparison of the predicted three-dimensional models of the catalytic domain of stetterlysin and pyrolysin with the crystal structure of subtilases from mesophilic and thermophilic origin, i.e. subtilisin BPN' and thermitase, and the homol. model of subtilisin S41 from psychrophilic origin, led to the identification of features that could be related to protein stabilization. Higher thermostability was found to be correlated with an increased no. of residues involved in pairs and networks of charge-charge and arom.-arom. interactions. These highly thermostable **proteases** have several extra surface loops and inserts with a relatively high frequency of arom. residues and Asn residues. The latter are often present in putative N-glycosylation sites. Results from modeling of known substrates in the substrate-binding region support the broad substrate range and the autocatalytic activation previously suggested for pyrolysin.

L5 ANSWER 9 OF 14 Elsevier BIOBASE COPYRIGHT 2003 Elsevier Science B.V.
ACCESSION NUMBER: 1997246025 Elsevier BIOBASE
TITLE: Methionine aminopeptidase from the hyperthermophilic archaeon pyrococcus furiosus: Molecular cloning and overexperssion in Escherichia coli of the gene, and characteristics of the enzyme
AUTHOR: Tsunasawa S.; Izu Y.; Miyagi M.; Kato I.
CORPORATE SOURCE: S. Tsunasawa, Biotechnology Research Laboratories, Takara Shuzo Co. Ltd., Kusatsu, Shiga 525, Japan. E-mail: s-tsunas@mx.biwa.or.jp
SOURCE: Journal of Biochemistry, (1997), 122/4 (843-850), 24 reference(s)
CODEN: JOBIAO ISSN: 0021-924X
DOCUMENT TYPE: Journal; Article
COUNTRY: Japan
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A gene for a methionine aminopeptidase (MAP; EC 3.4.11.18), which catalyzes the removal of amino-terminal methionine from the growing peptide chain on the ribosome, has been cloned from the hyperthermophilic Archaeon, Pyrococcus furiosus, by a novel method effectively using its cosmid protein library, sequenced and expressed in Escherichia coli. The DNA sequence encodes a protein containing 295 amino acid residues with methionine at the N-terminus. From protein analyses of the recombinant protein expressed in E. coli, by using both amino acid sequence analysis from the N-terminus by automated Edman degradation and analyses of molecular masses of the peptides generated by two enzymatic cleavages performed independently, digestions with lysylendopeptidase and Endoproteinase Asp-N, with ionspray mass spectrometry, the primary structure of the protein has been elucidated to be completely identical with that deduced from its DNA sequence. Comparison of the amino acid sequence of P. furiosus MAP (P.f. MAP) with those of other MAPs from Eukarya and Bacteria showed that the protein has a high degree of sequence homology in the stretches surrounding the five cobalt-binding residues fully preserved in all of MAPs determined so far, but P.f. MAP belongs to Type II because it has an extra long insertion of about 60 amino acid residues between the fourth and fifth cobalt-binding ligands, similar to MAPs from human and rat, and to Met-AP2 from Saccharomyces cerevisiae in comparison to Type I MAPs from Bacteria. Therefore, P.f. MAP seems to be rather close to those from Eukarya, although it is distinct in lacking the N-terminal extension of about 90-150 residues universally found in MAPs from Eukarya. These findings suggest that P.f. MAP is evolutionally located at the Eukarya-Bacteria boundary. The enzyme

expressed in E. coli exhibits a considerable thermostability, with a half-life of approximately 4.5 h at 90.degree.C and an optimum temperature of around 90.degree.C.

L5 ANSWER 10 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:441129 BIOSIS
DOCUMENT NUMBER: PREV199699163485
TITLE: Enzymes, high temperature.
AUTHOR(S): Adams, Michael W. W.
CORPORATE SOURCE: Dep. Biochemistry, Univ. Ga., Athens, GA 30602 USA
SOURCE: Meyers, R. A. [Editor]. (1996) pp. 240-249. Encyclopedia of molecular biology and molecular medicine, Vol. 2. Denaturation of DNA to growth factors.
Publisher: VCH Verlagsgesellschaft mbH Postfach 10 11 61, Boschstrasse 12, D-6940 Weinheim, Germany.
ISBN: 3-527-28472-9.
DOCUMENT TYPE: Book
LANGUAGE: English

L5 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 4
ACCESSION NUMBER: 1996:528382 CAPLUS
DOCUMENT NUMBER: 125:215332
TITLE: Isolation and characterization of the **hyperthermostable** serine **protease**, pyrolysin, and its gene from the hyperthermophilic archaeon Pyrococcus furiosus
AUTHOR(S): Voorhorst, Wilfried G. B.; Eggen, Rik I. L.; Geerling, Ans C. M.; Platteeuw, Christ; Siezen, Roland J.; de Vos, Willem M.
CORPORATE SOURCE: Department Microbiology, Wageningen Agricultural University, Wageningen, 6703 CT, Neth.
SOURCE: Journal of Biological Chemistry (1996), 271(34), 20426-20431
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **hyperthermostable** serine **protease** pyrolysin from the hyperthermophilic archaeon Pyrococcus furiosus was purified from membrane fractions. Two proteolytically active fractions were obtained, designated high (HMW) and low (LMW) mol. wt. pyrolysin, that showed immunol. cross-reaction and identical NH2-terminal sequences in which the third residue could be glycosylated. The HMW pyrolysin showwed a subunit mass of 150 kDa after acid denaturation. Incubation of HMW pyrolysin at 95.degree. resulted in the formation of LMW pyrolysin, probably as a consequence of COOH-terminal autoproteolysis. The 4194-base pair pls gene encoding pyrolysin was isolated and characterized, and its transcription initiation site was identified. The deduced pyrolysin sequence indicated a prepro-enzyme organization, with a 1249-residue mature protein composed of an NH2-terminal catalytic domain with considerable homol. to subtilisin-like serine **proteases** and a COOH-terminal domain that contained most of the 32 possible N-glycosylation sites. The archaeal pyrolysin showed highest homol. with eucaryal tripeptidyl peptidases II on the amino acid level but a different cleavage specificity as shown by its endopeptidase activity toward caseins, casein fragments including .alpha.S1-casein, and synthetic peptides.

L5 ANSWER 12 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:233009 BIOSIS
DOCUMENT NUMBER: PREV199698797138
TITLE: **Hyperthermostable** surface layer protein tetrabrachion from the archaebacterium Staphylothermus marinus: Evidence for the presence of a right-handed coiled

coil derived from the primary structure.

AUTHOR(S): Peters, Juergen (1); Baumeister, Wolfgang; Lupas, Andrei
CORPORATE SOURCE: (1) Max-Planck-Inst. Biochem., Am Klopferspitz 18a, D-82152 Martinsried Germany
SOURCE: Journal of Molecular Biology, (1996) Vol. 257, No. 5, pp. 1031-1041.
ISSN: 0022-2836.
DOCUMENT TYPE: Article
LANGUAGE: English

AB The scaffold of the surface layer covering the hyperthermophilic archaebacterium Staphylothermus marinus is formed by an extended filiform glycoprotein complex, tetrabrachion, which is anchored in the cell membrane at one end of a 70 nm stalk and branches at the other end into four arms of 24 nm length. The arms from a canopy-like meshwork by end-to-end contacts, enclosing a "quasi-periplasmic space". The primary structure of the complex, obtained by an approach based entirely on the polymerase chain reaction, shows that the light and the heavy chains are encoded in this order in a single gene and are generated by internal proteolytic cleavage. One light chain associates with the N-terminal part of a heavy chain to form one of the four arms of the complex, comprising about 1000 residues. Following a glycine-rich linker of about ten residues, the C-terminal 500 residues of the four heavy chains converge to form a four-stranded parallel coiled coil, which ends in a transmembrane segment. The sequence of the coiled coil is exceptional in that the heptad repeat of hydrophobic residues typical for left-handed coiled coils shifts to an undecad repeat after an internal proline residue, indicating that the C-terminal part of the sequence forms a right-handed coiled coil. Such a periodicity has not been detected in coiled coils to date. The almost flawless pattern of aliphatic residues, mainly leucine and isoleucine, throughout the hydrophobic core of the stalk provide one explanation for its exceptional stability.

L5 ANSWER 13 OF 14 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 5
ACCESSION NUMBER: 1996:385512 CAPLUS
DOCUMENT NUMBER: 125:108481
TITLE: A **hyperthermostable protease** of the subtilisin family bound to the surface layer of the Archaeon Staphylothermus marinus
AUTHOR(S): Mayr, Jutta; Lupas, Andrei; Kellermann, Josef; Eckerskorn, Christoph; Baumeister, Wolfgang; Peters, Juergen
CORPORATE SOURCE: Max-Planck-Institute Biochemie, Martinsried, D-82152, Germany
SOURCE: Current Biology (1996), 6(6), 739-749
CODEN: CUBLE2; ISSN: 0960-9822
PUBLISHER: Current Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A globular **protease** from the surface layer of Staphylothermus marinus, a marine archaeon with an optimum growth temp. of 92.degree., was purified and characterized with regard to its enzymic properties and thermostability. Its gene was sequenced using an approach based entirely on the polymerase chain reaction. The precursor form is 1345 amino acids long; between residues 64-741, it contains a domain with clear homol. to subtilisins, which is interrupted by 2 large insertions. The enzyme has a broad substrate specificity and a pH optimum of 9.0. It is fully stable from pH 3.2 to 12.7 and is resistant to heat-inactivation to 95.degree. in the free form and to 125.degree. in the bound form. This **protease** is one of the most stable **proteases** known. Despite its large size, it is clearly a member of the subtilisin family and represents the only known enzyme that is a stoichiometric surface layer component.

L5 ANSWER 14 OF 14 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 6
ACCESSION NUMBER: 1991:424850 CAPLUS

DOCUMENT NUMBER: 115:24850
TITLE: Properties of extremely thermostable **proteases** from anaerobic hyperthermophilic bacteria
AUTHOR(S): Klingeberg, Michael; Hashwa, Fuad; Antranikian, Garabed
CORPORATE SOURCE: Arbeitsbereich Biotechnol. I, Tech. Univ. Hamburg-Harburg, Hamburg, D-2100/90, Germany
SOURCE: Applied Microbiology and Biotechnology (1991), 34(6), 715-19
CODEN: AMBIDG; ISSN: 0175-7598
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Hyperthermostable proteases** were characterized from five archaebacterial species (Thermococcus celer, T. stetteri, Thermococcus strain AN1, T. litoralis, Staphylothermus marinus) and the hyperthermophilic eubacterium Thermobacteroides proteolyticus. These **proteases**, which were found to be of the serine type, exhibited a preference for phenylalanine in the carboxylic side of the peptide. The enzymes from T. stetteri and T. litoralis hydrolyzed most substrates (peptides) tested. All **proteases** were extremely thermostable and demonstrated optimal activities between 80 and 95.degree.. The pH optimum was either neutral (T. celer, Thermococcus strain AN1) or alk. The **protease** of T. proteolyticus was optimally active at pH 9.5. Zymogram staining showed the presence of multiple **protease** bands for all strains investigated.